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While a strong rationale for chemoprevention of ovarian carcinoma exists, a mechanism for the comprehensive evaluation of novel compounds is severely impeded by the lack of a validated animal model of spontaneous ovarian carcinogenesis. At present, there is no verified, established model for this disease. In rodents, this type of cancer does not spontaneously develop. While studies investigating "induced" carcinomas have been performed they are hindered by biologic differences in induced and spontaneous tumor formation. Identification of spontaneous ovarian carcinogenesis in the laying hen (*Gallus Domesticus*) may provide the answer to this dilemma. Multiple reports have demonstrated a 30-50% rate of spontaneously arising genital tract adenocarcinomas in hens of 3-6 years of age. Thus, the purpose of this study will be to utilize this animal model to evaluate the ability of a COX-2 inhibitor to reduce the incidence of spontaneous ovarian carcinogenesis in this animal model. More importantly, identification of promising agents in surrogate animal models that simulate a high risk population would significantly impact the strategy of cancer chemoprevention for ovarian carcinoma and lead to subsequent endeavors in this neglected area of study.

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ANNUAL REPORT: CONTROLLED TRIAL OF CHEMOPREVENTION USING COX-2 INHIBITORS IN AN AVIAN MODEL OF SPONTANEOUS OVARIAN CARCINOGENESIS (W81XWH-04-1-0322)

INVESTIGATORS: Mack N. Barnes MD, Wallace D. Berry PhD

INTRODUCTION

Ovarian carcinoma represents the fourth leading cause of cancer death in the female population and is the most fatal gynecologic malignancy. Secondary to its presentation as advanced disease in the majority of cases and its low prevalence, strategies that center on therapeutics or screening are unlikely to impact the overall death rate. Therefore, when considering strategies to decrease the deaths attributable to ovarian carcinoma, prevention of disease represents the most rational approach. This model of intervention is consistent with statements from the National Cancer Institute which have identified a need for development of chemoprevention strategies in ovarian carcinoma. Additional support of ovarian cancer chemoprevention is the historical benefit of oral contraceptives and retinoic acid derivatives. A critical component of developing chemopreventive strategies in ovarian carcinoma is identification of promising new compounds in validated animal models of spontaneous ovarian carcinogenesis. As will be detailed subsequently, a novel avian model utilizes the laying hen (*Gallus Domesticus*) and exploits the high rate of spontaneous ovarian carcinogenesis observed in this avian species. **Therefore, our hypothesis is that administration of a COX-2 inhibitor will result in a decreased rate of development of ovarian carcinoma in an avian model of spontaneous ovarian carcinogenesis.** The specific aims and methods pursuant to this proposal are as follows: Specific Aim#1: Determine the maximally tolerated dose (mg/kg) of COX-2 inhibitor, admixed with standard feed, in the laying hen. The effect of varying doses of COX-2 inhibitor on egg-laying activity as a surrogate marker for ovulatory frequency will also be assessed. The maximally tolerated dose will then be used in the controlled trial. Specific Aim#2: Determine, in a controlled chemoprevention trial, the ability of a COX-2 inhibitor to inhibit the development of spontaneously arising genital tract adenocarcinoma in the laying hen. These studies will address an important and underdeveloped investigational endeavor in women's health. Clearly, chemoprevention represents the most rational investigational strategy to achieve a meaningful reduction in deaths from ovarian carcinoma. More importantly, identification of promising agents in surrogate animal models that simulate a high risk population would significantly impact the strategy of cancer chemoprevention for ovarian carcinoma and lead to subsequent endeavors in this neglected area of study. As detailed subsequently, and in accordance with the timeline proposed in the original statement of work, the hens were acquired, the dose finding and toxicity study completed and the controlled trial initiated.

BODY

As noted above the statement of work included 2 specific aims: Specific Aim#1: Determine the maximally tolerated dose (mg/kg) of COX-2 inhibitor, admixed with

standard feed, in the laying hen. The effect of varying doses of COX-2 inhibitor on egg-laying activity as a surrogate marker for ovulatory frequency will also be assessed. The maximally tolerated dose will then be used in the controlled trial. Specific Aim#2: Determine, in a controlled chemoprevention trial, the ability of a COX-2 inhibitor to inhibit the development of spontaneously arising genital tract adenocarcinoma in the laying hen. The accomplishments toward the successful completion of each specific aim will be addressed individually.

Specific Aim#1: Determine the maximally tolerated dose (mg/kg) of COX-2 inhibitor, admixed with standard feed, in the laying hen. The effect of varying doses of COX-2 inhibitor on egg-laying activity as a surrogate marker for ovulatory frequency will also be assessed. The maximally tolerated dose will then be used in the controlled trial.

There is a paucity of information regarding the use of COX-2 inhibitors in the avian hen. Rimadyl (Pharmacia Inc.) is a COX-2 specific inhibitor approved for use in veterinary medicine and available in a stable powder form. Therefore, in accordance with specific aim #1 a dose/toxicity study was performed prior to initiation of the larger study. This study was carried out in accordance with plans outlined in the original proposal.

Three dose levels were established as follows:

Control: 0 mg/kg body weight
Dose 1: 2.5 mg/kg body weight
Dose 2: 5 mg/kg body weight
Dose 3: 10 mg/kg body weight

Rimadyl was added with other feed ingredients at the time feed was mixed in the feed mill and delivered to the hens. 5 hens were utilized per dose level for a total of 20 hens. The hens were monitored for egg laying activity as defined by percent egg laying activity control (0 mg/kg) and egg shell quality. In addition, toxicity will be monitored based on general appearance, feather quality, evidence of gastrointestinal bleeding, and death. Over an 8 week study period, no toxicity and no lethal events were noted. In addition, there did not appear to be any effect on ovulatory activity, even at the highest dose level. Given, the ability of these hens to tolerate the highest dose level, 10mg/kg body weight was determined to be the dose we would utilize in the controlled trial.

Specific Aim#2: Specific Aim#2: Determine, in a controlled chemoprevention trial, the ability of a COX-2 inhibitor to inhibit the development of spontaneously arising genital tract adenocarcinoma in the laying hen.

As outlined in the proposal, a power calculation had been performed using assuming a 40% incidence of genital tract adenocarcinoma. It was estimated that to identify a reduction in disease of 20% at the 0.05 level of significance a treatment group of 88 hens and a control group of 88 hens would be required. The informative cases defined as those hens completing the course of treatment (as previously used in a published study. Significant attrition from non-malignant causes is known to occur as hens age. Therefore, to initiate this study 480 hens (*Gallus Domesticus*) were acquired 8/3/04.

This allowed the establishment of a treatment group (240 hens) and a control group (240 hens) that should account for attrition. It is our practice to allow a period of time for hens to "acclimate" and, therefore, a period of 8 weeks was utilized to allow the hens to acclimate. The controlled study was then initiated October 1, 2004 using a daily dose of Rimadyl of 10mg/kg body weight. This trial is ongoing at the date of this report and planned for 48 months.

KEY RESEARCH ACCOMPLISHMENTS

- Dose Finding Study completed
- No toxicity demonstrated for Rimadyl (COX-2 inhibitor) at 10mg/kg body weight
- Hens acquired for trial and acclimated
- Controlled trial initiated 10/04

REPORTABLE OUTCOMES.

Too early.

CONCLUSIONS

Too early.

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